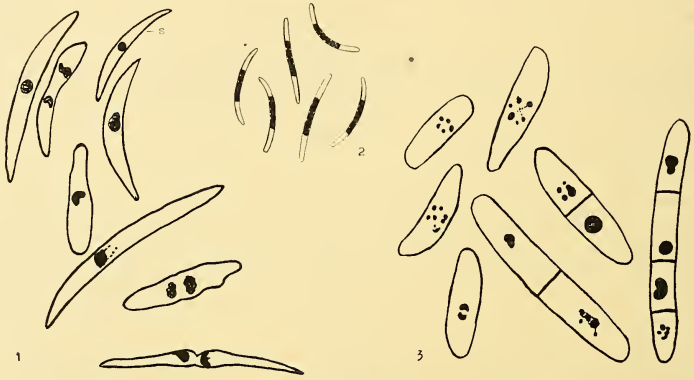
Selenophoma Species.

Mature spores examined of *S. donacis* and *S. donacis* var. *stomaticola* had one nucleus per spore (Plate vii, 8).

Conidia of the variety are produced abundantly in culture, and more or less retain the falcate shape. In some spores a septum is laid down at the centre, and in these two nuclei—or one per cell—occurred. Conidia of the species are also produced abundantly in culture, but vary from falcate to linear to sausage-shaped, together with many other abnormal forms. In the falcate-shaped spores the nuclei stained vividly and were regularly rounded in outline, with the cytoplasm only faintly stained. In the abnormally-shaped spores the nuclei were more difficult to differentiate. In dividing spores two nuclei occurred, or one per cell (Text-fig. 1).



Text-figures 1-3.

1. Conidia of *Selenophoma donacis* from culture, stained with Giemsa, showing one nucleus per spore and two nuclei in dividing spores. Spore "s" is similar to pycnidiospores from the field. ×1000.

2. Micropycnidiospores of *Septoria tritici*, stained with Giemsa, showing one linear, slightly "beaded" nucleus per spore. ×2000.

3. Single-celled immature spores of *Septoria nodorum* pressed out of pycnidia, showing one region of nuclear activity per cell: three cells with 5-7 fragments and one cell with a nucleus of two parts. Some nuclei in the more mature 2- and 4-celled spores also showing fragments. Stained with Giemsa. ×2000.

Other Genera.

Spores of *Colletotrichum* sp., *Glocosporium* sp. and *Phyllosticta* sp. showed one nucleus per cell, i.e., one nucleus per spore, when stained with Giemsa. Spores of *Ascochyta* sp. also had one nucleus per cell, or two per spore, and spores of *Fusarium* sp. had one nucleus per cell, so that the microspores had one per spore, and the macrospores had the same number of nuclei as the number of cells in the spores. Conidia of *Neurospora tetrasperma* had from several to many (exact number not determined) nuclei per cell, and the mycelium had many approximately round nuclei scattered throughout the cells (Plate vii, 7).

Discussion.

The Feulgen reaction is specific for desoxyribonucleic acid, and Murray *et al.* (1950) and Tulasne and Vendreley (1947) stated that the Giemsa stain may also be considered to demonstrate the distribution of DNA (desoxyribonucleic acid). DNA is concentrated in the nucleus and there is little doubt, therefore, that the areas stained in these preparations with Giemsa do represent the nuclei.

Some workers have recorded that certain macroconidia contain more than one nucleus per cell, e.g., the conidia of *Neurospora crassa*, where 1–20 are common (Barratt

and Garnjobst, 1949), and in *Helminthosporium carbonum*, where each cell of the 1- to 9-celled mature conidia contains from 1–8 nuclei (Roane, 1952).

The present study has shown, however, that the macro- and micropycnidiospores and conidia of the species of *Septoria* and *Selenophoma* examined have one nucleus per cell. From the evidence obtained from the one-celled immature spores of *Septoria nodorum*, where only one area of nuclear reaction was detected, it would seem that the nuclei in the mature spores are all derived from one nucleus.

The nuclei of the spores of *S. nodorum* can be resolved into 5–7 fragments. This might be an artefact produced by the methods used, or might truly represent 5–7 chromatinic areas carrying a heavier charge of desoxyribonucleic acid.

Darlington and La Cour (1940) pointed out that with *Trillium* the over-nucleated chromocentres of the resting stage are in fact the under-nucleated differential segments of metaphase—they are the heterochromatic parts of the chromosomes. Hillary (1939), in tests with the Feulgen reaction, using tissue of animals, plants, bacteria and fungi, recorded that with fungi (species of *Mucor*, *Geopyxis* and *Aleurodiscus*) there was in most cases a large nucleus with small chromocentres distributed around the nucleolus and the periphery of the nucleus.

As the actual division of the nucleus into two was not observed in *S. nodorum*, it is impossible to say whether the 5–7 fragments retain their identity in the actively dividing state, or whether they are of a heterochromatinic nature and are undercharged with DNA when the nucleus divides.

Elongated nuclei were usual in these preparations of germ tubes and hyphae. Smith (1923) noted "long torpedo-like" nuclei in some parts of the thallus of *Saprolegnia*, and Wilson (1937) stated that in the spongy framework of the sporophore of *Peziza rutilans* were "hyphae taking an unusual straight course with septa at infrequent intervals and long spindle-shaped nuclei pressed in single file against their walls. So peculiar did these nuclei appear that some doubt was felt as to their nature until the Feulgen reaction was carried out, when the chromatin threads were brightly coloured. The elongation of the nuclei does not appear to be caused by the narrowness of the hyphae as is the case in the paraphyses."

Smith (1923) considered that the constant upward streaming seemed to cause a tension or strain within the semi-liquid cytoplasm, and the nuclei responded to the strain by becoming elongated.

It is considered that the linearity of the nuclei of the microspores of *S. tritici* is due to the conformation of the microspore. The elongated nuclei in the hyphae and germ tubes might also be caused by the narrowness in relation to the size of the nuclei.

When the linear nuclei were first observed, it was thought that the linearity might have been caused by the methods of drying and fixing used, but the condition persisted when the speed of drying was altered by varying the temperature and when fixations were carried out without previous drying. It is also to be noted that rounded nuclei in spores occurred in the same preparations as linear nuclei in hyphae and microspores. Also, in the conidia and hyphae of *Neurospora tetrasperma*, the nuclei, which are small in relation to the cells containing them, are revealed by using Method 1 to be nearly circular, and are similar in appearance to those figured by Cutter (1946) using a completely different technique.

The linear nuclei in the microspores and in many of the hyphae have a "beaded" appearance. These "beads" might represent the fragments seen in some of the nuclei of the spores.

The difference in the intensity of stain in the nuclei in germinated and ungerminated spores probably indicates a change in the distribution of the desoxyribonucleic acid as the cell begins to germinate.

Stained spores were examined in every stage of germination, and it was noteworthy that no nucleus in the many germinating *Septoria* spores examined was detected in the act of dividing—the nuclei in young germ tubes all appeared at some little distance from the spore nuclei. The closest observed was in the spore shown in Plate vii, 5.

Details of mitosis in dividing spores of *Selenophoma* could not be determined. The nuclei had an amitotic appearance (Text-fig. 1), but as pointed out by Cutter (1946) for other fungal nuclei, this might be an artefact.

In an endeavour to observe the mitotic division, living spores of both *Septoria* spp. and *Selenophoma* sp. were kept under continuous observation under phase contrast, but, as already noted, no nucleus could be detected, either in phase or with dark field. This confirms the finding of McMillan and Plunkett (1942), who, using bright and dark field microscope, could find no structure that could be construed as a nucleus. Apparently the R.I. of the nucleus in these spores is so similar to the R.I. of the cytoplasm that it cannot be detected even with phase contrast, or else the cytoplasm is so dense that the nucleus is obscured.

2. Fat Reaction.

Spores of *Septoria*, *Selenophoma* and *Ascochyta* from the field and from culture were stained to demonstrate the amount of fat present and its distribution.

Spores were allowed to exude from pycnidia into water on clean slides, or secondary spores were added to the water from culture and allowed to air-dry. Spores adhered to the slides during all the subsequent treatments. Fat was stained according to the methods outlined below.

Methods.

Method 1. Sudan III.

A saturated solution of Sudan III in 70% alcohol and pure acetone (1:1) was prepared, and the spores on the slides treated as follows (after Conn, 1936): (i) Fix in the vapour of formaldehyde for 10 minutes; (ii) stain in Sudan III for 10 minutes (in a sealed dish); (iii) dip for an instant in 65% alcohol; (iv) wash in water; (v) mount in glycerine.

Method 2. Sudan IV.

A saturated solution was prepared in 70% alcohol and the material treated as in Method 1.

Both Sudan III and Sudan IV stained fat a vivid orange. Cotton-blue was sometimes used as a counter stain.

Spores were also treated with benzol or ether, either (*a*) before the above staining treatments, to remove the fat (no fat was detected after the subsequent staining treatments); or (*b*) after staining. In this case the stained fat disappeared very slowly.

Fat can also be demonstrated in spores by treating with cotton-blue without previous air-drying or staining. Fat globules remain unstained.

Results.

Filiform Septoria *Spores, as* Septoria tritici.

Usually no large guttulae are visible in unstained spores from the field, and, when viewed at high magnifications, only small guttulae in spores stained with cotton-blue.

After staining with Sudan, a fat reaction can be detected by a faint orange "speckled" condition over the spore, and very occasionally in very small globules, often near the septa.

In culture many of the species produce conidia directly on the mycelium. The conidia vary in shape from symmetrically filiform to asymmetrically bacillar, with a varying number of septa. When grown on P.D.A. an abundance of fatty material can be detected in most conidia, especially from old cultures. It occurs first as fatty globules strung along the spore, later forming patches or blocks, sometimes nearly occupying the whole spore, which loses its identity. It stains a vivid orange colour with Sudan.

When counterstained with cotton-blue lacto-phenol, the orange colour remains undisturbed, as if the whole interior of that portion of the cell were a block of fat.

Cylindrical Septoria *Spores, as in* S. nodorum, S. avenae.

In the unstained spores from the field, and in spores stained with cotton-blue lacto-phenol, large and small guttulae, sometimes nearly the width of the spore, can easily be detected. They are usually situated at both ends of the cells, i.e., clustered around the septa and at the ends of the spores.

Staining with Sudan colours the guttulae a deep red-orange. The rest of the cell remains unstained—no "speckling" occurs as in the filiform spores (Plate vii, 10).

These species do not, as far as is known, produce conidia in culture, but occasionally pycnidiospores are produced. When stained with Sudan the spores show fat accumulation in all stages, from those with guttulae of various sizes clustered at each end of the cells around the septa, to those where the numbers of guttulae have increased and spread from the ends towards the centre of the cells. At a later stage practically the whole spore, except for the septa and an area about the centre of each cell, is coloured a deep orange. At a high magnification this fat is revealed as masses of rather evenly-sized globules. The free area in the centre is probably that occupied by the nucleus.

Ascochyta *sp. from* Bromus unioloides.

Fat distribution in these spores is similar to that in the cylindrical type of *Septoria* spore, where guttulae are clearly visible in the unstained and cotton-blue stained spores from the field. After treatment with Sudan, the guttulae stain a deep orange, with the rest of the spore unstained (Plate vii, 9).

Selenophoma *sp.*

No guttulae were visible in the spores of most field collections of *Selenophoma*, either unstained or stained with cotton-blue. With these spores, either no fat, or a faint "speckling" towards the ends of the spore, was detected with Sudan.

As with the filiform type of *Septoria* species, secondary spores are produced directly on the mycelium in culture. When treated with Sudan, well-formed symmetrical young spores gave no fat reaction. Other spores, older and more asymmetrical, had faint pale orange "speckling", and some had a few circular globules which gave a deep orange colour. Old and knobbly mycelium from culture was packed with large globules and stained vividly with Sudan.

Discussion.

These tests show that the amount of fat, judged qualitatively, and its distribution, is different in the two types of *Septoria* spores as they occur in the field. Spores of *Ascochyta* sp. from *Bromus unioloides* gave a reaction for fat similar to the cylindrical type of *Septoria* spores, and spores of *Selenophoma* gave a reaction similar to the filiform type of *Septoria* spores. Under cultural conditions favouring high fat synthesis, additional fatty material is stored in the cells to such an extent that very large globules or whole blocks of fat occur, particularly in the filiform spores.

Foster (1949) has pointed out that with fungi, while the major deposits of fat obviously are in vacuole globules, some lipide material undoubtedly does exist in the cytoplasm proper, and some fatty materials are laid down in the cell wall of fungi. In this latter case the fatty material is sometimes protected.

Slight differences were detected in the intensity of the stain in some of the spores, particularly in those of *Ascochyta* sp. The colour varied from bright orange to deep orange. Sudan is reputed to colour "true fats" intensely, and cholesterin esters and cholesterin-fatty acid mixtures less intensely. The slight differences in the intensity of the above preparations could be due to differences in the type of fat present, or to differences in the concentration of the fat in the globules.

Acknowledgements.

The writer is indebted to Professor W. L. Waterhouse and to various members of the Faculty of Agriculture and School of Botany, Sydney University, for comment and advice, and to Mr. S. Woodward-Smith for some of the photographs.

References.

ALLISON, J. LEWIS, 1945.—*Selenophoma bromigena* leaf spot on *Bromis inermis*. *Phytopath.*, 35 : 233-240.

BARRATT, R. W., and GARNJOBST, L., 1949.—Genetics of a colonial microconidiating mutant strain of *Neurospora crassa*. *Genetics*, 34 : 351-369.

CHEREWICK, W. J., 1944.—Studies on the biology of *Erysiphe graminis* D.C. *Canad. Jour. Res.*, 22 (C) : 52-86.

CONN, H. J., 1936.—Biological Stains. Geneva, U.S.A.

CUTTER, V. M., 1946.—The chromosomes of *Neurospora tetrasperma*. *Mycol.*, 38 : 693-698.

DARLINGTON, C. W., and LA COUR, L., 1940.—Nucleic acid starvation of chromosomes in *Trillium*. *Jour. Gen.*, 40 : 185-213.

———, 1947.—The handling of chromosomes. London. 2nd Edition.

FOSTER, J. W., 1949.—Chemical activities of fungi. New York.

HILLARY, B. B., 1939.—Use of the Feulgen reaction in cytology. 1. Effect of fixative on the reaction. *Bot. Gaz.*, 101 : 276-300.

KNAYSI, G., HILLIER, J., and FABRICANT, C., 1950.—The cytology of an avian strain of *Mycobacterium tuberculosis* studied with electron and light microscopes. *Jour. Bact.*, 60 : 423-447.

MACNEILL, BLAIR H., 1950.—Studies in *Septoria lycopersici* Speg. Abstr. *Phytopath.*, 40 : 18.

MCCLINTOCK, B., 1945.—*Neurospora*. 1. Preliminary observations of the chromosomes of *Neurospora crassa*. *Amer. Jour. Bot.*, 32 : 671-678.

MCMILLAN, H. G., and PLUNKETT, O. A., 1942.—Structure and germination of *Septoria* spores. *Jour. Agr. Res.*, 64 : 547-559.

MOORE, W. C., 1940.—New and interesting plant diseases. *Trans. Brit. Mycol. Soc.*, 24 : 345-351.

MURRAY, R. G. E., GILLEN, D. H., and HEAGY, F. C., 1950.—Cytological changes in *Escherichia coli* produced by infection with phage T2. *Jour. Bact.*, 59 : 603-615.

ROANE, C. W., 1952.—Nuclear cytology and morphologic variation in *Helminthosporium carbonum* Ullstrup. Abstr. *Phytopath.*, 42 : 480.

ROBINOW, C. F., 1944.—Cytological observations on *Bacterium coli*, *Proteus vulgaris* and various aerobic spore forming bacteria with special reference to the nuclear structures. *Jour. Hygiene*, 43 : 413-423.

SHAW, D. E., 1951.—A Septoria disease of *Euphorbia peplus* L. PROC. LINN. SOC. N.S.W., 76 : 7-25.

———, 1952.—Ropy smut of Liverpool Plains grass. PROC. LINN. SOC. N.S.W., 77 : 142-145.

SMITH, FRANCIS E. V., 1923.—On direct nuclear divisions in the vegetative mycelium of *Saprolegnia*. *Anal. Botany*, 27 : 63-73.

SPRAGUE, R., 1934.—A physiologic form of *Septoria tritici* on oats. *Phytopath.*, 24 : 133-143.

TULASNE, R., and VENDRELEY, R., 1947.—Demonstration of bacterial nuclei with ribonuclease. *Nature*, 160 : 225-226.

VANTERPOOL, T. C., 1947.—*Selenophoma linicola* sp. nov. on flax in Saskatchewan. *Mycol.*, 39 : 341-348.

WILSON, I. M., 1937.—A contribution to the study of the nuclei of *Peziza vutilans* Fries. *Ann. Bot.*, N.S., 1 : 655-672.

EXPLANATION OF PLATE VII.

1. Spores of *Septoria nodorum* stained with Giemsa. The nuclei (one per cell) are vividly stained, the septations clear, and guttulae are visible in the cells as unstained circular areas. ×1000.

2. Micropycnidiospores and portion of a macropycnidiospore of *Septoria tritici* stained with cotton-blue lacto-phenol. ×1000.

3. Micropycnidiospores and portions of small macropycnidiospores of *Septoria tritici* from summer material from the field, stained with Giemsa. Note the linear nucleus in each microspore, conforming to the shape of the spore, and the rounded nuclei in the macrospores. The cytoplasm in the ends of the microspores is only faintly stained. ×1000.

4. Filiform spores of *Septoria lactucae* stained with Giemsa, showing one nucleus per cell and faint septations. ×1000.

5. Germinating spore of *Septoria avenae* stained with Giemsa, showing germ tubes from three cells. The four nuclei in the spore are vividly stained, the linear nuclei in the germ tubes less intensely stained. Photograph by Woodward-Smith. ×900.

6. Hyphae of *Septoria* sp. from *Anthoxanthum odoratum* from culture, stained with Giemsa, showing linear nuclei. Photograph by Woodward-Smith. ×900.

7. Conidia of *Neurospora tetrasperma* stained with Giemsa, showing many small rounded nuclei per spore, the spore walls being out of focus. Photograph by Woodward-Smith. ×900.

8. Spores of *Selenophoma donacis* var. *stomaticola* from culture, stained with Giemsa, showing one nucleus per spore. ×1000.

9. Spores of *Ascochyta* sp. from the field, stained with Sudan, showing heavily stained fat globules clustered at each end of the cells. Photographed with a blue filter. ×1000.

10. Spore of *Septoria nodorum* from the field, stained with Sudan, showing heavily stained fat globules distributed as in the *Ascochyta spores*. Photographed with a blue filter. ×1000.

THE *CULEX PIPIENS* GROUP IN SOUTH-EASTERN AUSTRALIA. II.

By N. V. Dobrotworsky, Georgina Sweet Fellow in Economic Entomology, and F. H. Drummond, Zoology Department, University of Melbourne.

(Five Text-figures.)

[Read 29th July, 1953.]

Synopsis.

The *Culex pipiens* complex in Australia consists of three forms: *C. fatigans, C. pipiens* form *molestus* and *C. pipiens australicus,* n. subsp. An account is given of their morphological and biological characteristics, their distribution in Australia and their capacity for interbreeding. These observations provide the basis for a discussion of the taxonomic status of the three forms.

In its morphology and biology the Australian *molestus* conforms to *C. molestus* as described by Marshall and Staley. The status of this mosquito remains obscure and until its relationships to *C. pipiens* and *C. fatigans* are more definitely established, it should be called *C. pipiens* form *molestus.* It is recorded from Victoria and northern Tasmania.

C. fatigans is widely distributed in Australia but in southern Victoria it is found regularly only in late summer and autumn. It hybridizes freely with *C. pipiens* form *molestus* but no permanent populations of intermediates have been found in Victoria. Interbreeding between *C. fatigans* and other members of the *C. pipiens* complex has been recorded from various parts of the world but the available evidence does not seem to justify the reduction of *C. fatigans* to the status of a subspecies of *C. pipiens.*

C. pipiens australicus, n. subsp., is also widely distributed in Australia. Morphologically it is distinct from other members of the complex; biologically it is very similar to *C. pipiens.* It is a rural non-man-biting mosquito which is anautogenous, eurygamous and heterodynamic. It has a limited capacity for interbreeding with *C. fatigans* and *C. pipiens* form *molestus* in the laboratory but in nature is reproductively isolated from both these forms.

Introduction.

The problems presented by the *Culex pipiens* complex (Mattingly *et al.,* 1951) concern the relationships of *C. pipiens* L., *C. fatigans* Wied. and *C. molestus* Forskål.

Until recently the status of *C. pipiens* and *C. fatigans* as distinct species had not been seriously questioned, but there is now evidence from various parts of the world, and particularly from the United States, that where the two forms occur together, they interbreed with the production of permanent populations of intermediates. Hence it has been claimed that *fatigans* should be treated as a subspecies of *C. pipiens* L. It is however, not clear that the mosquito involved in these hybridizations is *C. pipiens,* s.s.; in some cases there is no doubt that it is actually *C. molestus.*

C. molestus was described by Forskål in 1778 but subsequently was included in the synonymy of *C. pipiens* L. In 1937 it was again recognized as a distinct species by Marshall and Staley (1937). Over a period of some years the observations of a number of workers had indicated the existence of two biological races of *C. pipiens* in Europe. One was a man-biting form which was autogenous, stenogamous and homodynamic; the other was anautogenous, eurygamous and heterodynamic and did not attack man. Marshall and Staley (1937) claimed that the two forms presented constant morphological differences and should be regarded as distinct species. For the autogenous form they revived Forskål's name *C. molestus*; the name *C. pipiens* L. they restricted to the anautogenous one.

This conclusion has not been universally accepted; some authors follow Marshall and Staley, but others regard *molestus* as a subspecies, or merely as a biotype, of *C. pipiens.* Thus the name *pipiens* as used by some authors, including nearly all the earlier ones, has a wide meaning, as used by others, a narrow one. In order to avoid confusion we shall use the terms *pipiens* and *molestus* in the sense in which they were defined by Marshall and Staley (1937).

The *C. pipiens* complex in Australia consists of three forms: *fatigans*, *molestus* and a third form which, as far as is known, is confined to this country. We regard this form as a new subspecies of *C. pipiens* L. Prior to its formal description, which cannot appropriately be given until its relationships to the other members of the complex have been discussed, we will refer to it as *australicus*.

A. Morphological and Biological Characteristics of the Members of the Complex.

a. fatigans.

The form *fatigans* has a world-wide distribution in the tropics and subtropics and is the common domestic *Culex* over the greater part of Australia. In southern Victoria, however, it seems unable to maintain itself permanently. Drummond (1951) stated that in some years it was rare or absent in Melbourne, but detailed observations during 1951–52 indicate that its disappearance is a seasonal phenomenon. During the autumn of 1951 it was abundant in Melbourne but in the following spring could not be found. It was present in small numbers in January, 1952, at which time the other members of the *pipiens* group were abundant. It increased steadily during February, and in March the larvae were very numerous in all kinds of artificial water containers. Oviposition continued freely until the end of May and on a small scale for another month. However, most of the larvae emerging from eggs laid late in May and in June died before the end of July. A few pupated during the winter and adults emerged from time to time—one emergence was recorded in late August—but apparently they did not establish themselves. Thus in two successive years *fatigans* was abundant during the autumn but rare or absent in the spring.

C. fatigans is homodynamic and is said to be incapable of hibernation. We have not found hibernating adults but this is not significant, as we have likewise failed to find hibernating *australicus*, a form which is certainly able to hibernate. In Melbourne, reproduction in *fatigans* is brought to an end by winter temperatures and even if the adults emerging in June were able to survive the winter they would not have been fertilized because the low night-temperatures of autumn and early winter would inhibit mating. In the laboratory mating will not occur at temperatures below 20°C. Males would not be expected to survive, since they do not do so even in species which are known to hibernate. Resumption of breeding in the spring would then depend upon the survival of adults emerging from the small winter population of pupae; *fatigans* would thus be rare or absent during early spring. This difference from *molestus*, which is also homodynamic, can be attributed to the higher temperature requirements of *fatigans*.

A morphological characteristic of *fatigans* which requires comment here is the siphon index of the larva. Woodhill and Pasfield (1941) gave the index for Australian *fatigans* as ranging from 3·4 to 6·5. It seems that their material included larvae of *australicus* which at that time had not been distinguished from *fatigans*.* In collections from several localities in Victoria the index for *fatigans* larvae never exceeded 4·8 (Table 1).

The number of branches on head-seta *f* varies from two to six with a mean of five. This is greater than the number given by Hopkins (1936). This seta is of no value in distinguishing *fatigans* from the other members of the *pipiens* group in Australia.

b. molestus.

The form *molestus* was first recorded from Australia by Drummond (1951). At that time it was known from southern Victoria up to sixty miles north of Melbourne but its range now extends to the northern border of the State (Mildura, Albury), and southwards to Tasmania. Although Mattingly (1951, 1952) has described *molestus* as an urban mosquito it is not restricted to urban situations in Victoria. Here it is common in rural areas in the vicinity of dwellings.

* The larvae of *australicus* were first recognized as distinct from typical *fatigans* by Dr. E. N. Marks in 1942. In correspondence she referred to them as "long-siphoned *fatigans*".

Morphologically, Australian *molestus* is indistinguishable from the European as described by Marshall and Staley (1937). The general colour is pale, the basal tergal bands are not constricted at the sides, and the venter is clothed entirely with pale scales. Some specimens collected in the autumn were darker than usual and had the general colour of *pipiens*. However, the venter was without dark scales and apart from the darker colour these specimens retained all the characteristics of *molestus*.

In the female the first fork cell is long (Table 2); the ratio of cell to petiole varies from 4·4 to 8·5, with a mean of 5·2. In the male the combined length of the first four segments of the palps is less than the length of the proboscis. The dimensions of the palps correspond closely with those given by Christophers (1951) (Table 3). The hypopygium, which is identical with that of the European *molestus*, will be discussed later.

The larvae also agree with the descriptions given by Marshall and Staley (1935) and Jobling (1938). The siphon index varies from 3·3 to 4·9, with a mean of 4·3.

TABLE 1.

Siphon Index of fatigans *from Victoria. Measurements are Expressed in Microns.*

Locality.	No.	Siphonal Index.			Length of Siphon.		
		Max.	Min.	Mean.	Max.	Min.	Mean.
Merbein—horse trough ..	50	4·6	3·5	4·3	1350	1098	1224
Merbein—rain-water tank ..	48	4·8	4·0	4·4	1384	1206	1296
Merbein—goose pond ..	50	4·6	3·6	4·2	1332	1026	1206
Culgoa—pool	49	4·6	3·7	4·0	1546	1260	1368
Melbourne	53	4·8	4·0	4·3	1530	1260	1296
	250	4·8	3·5	4·2	1546	1026	1278

C. molestus is a stenogamous mosquito; mating will occur in a space of a few cubic inches. In larger cages males may mate with resting females, but more usually mating is initiated while both sexes are in flight and is completed on the floor of the cage. In nature, swarming of males was often observed. It occurs just after sunset, between buildings or above the surface of water in tanks or butts. The swarms consisted of ten to thirty males.

A characteristic which has been regarded as highly distinctive of *molestus* is its capacity for autogenous reproduction. It is now known that in crosses, autogeny behaves as a simple mendelian recessive and it seems that the gene in question is not limited to *molestus* (Laven, 1951); in some populations of *molestus* it may be rare: in Cairo, Knight and Malek (1951) found that only one to four per cent. of females in wild populations were autogenous. Our earlier observations had indicated that a high proportion of Australian *molestus* were autogenous but, as Mattingly has pointed out, such a conclusion could have been influenced by unconscious selection in a laboratory colony. However, in the course of a recent experiment a group of thirty-nine females reared from a natural population of pupae produced thirty-eight autogenous egg rafts. Further work on the frequency of autogeny is in progress.

Several workers have noted that with *molestus* the egg rafts laid after a blood meal are generally larger than those produced autogenously. The size of the raft is also influenced by the size of the mosquitoes. A group of females which, because of an unfavourable larval environment, were below normal size and which were fed on human blood, laid rafts containing 50–60 eggs. On the other hand, autogenous rafts from females of normal size may contain 120–130 eggs. In rafts collected at natural breeding places the number of eggs varied from 30 to 178; in the majority the number was 70–125. The rafts are variable in shape; they may be oval, triangular or elongate.

In the laboratory *molestus* will breed without interruption throughout the year. In colonies maintained in outdoor cages emergences of adults, and egg-laying, continued

during June and into the early part of July. In natural breeding places also, egg rafts were plentiful until the end of June and during one mild spell (temperature 14°C.) dancing of males was observed. It was noted, however, that attacks on man ceased about the middle of May. This was perhaps due to low night temperatures; it suggests that during the late autumn *molestus* maintains itself largely by autogeny.

Larvae which hatched from eggs laid in outdoor cages in June passed the winter in the third or fourth stage. The majority of larvae hatching in July died; the survivors reached the third stage in August. Emergence of adults from these colonies and from exposed natural breeding sites commenced in September but in some sheltered places, such as drainage pits, pupae were present during the winter and emergence was complete by the end of August. There is therefore no hibernation; Australian *molestus*, like the European, is homodynamic.

It is a man-biting mosquito and in Melbourne is a troublesome pest. It enters houses and bites at night. In this respect it is active from October until May.

Larval Ecology.—Occasionally, and mainly in the autumn, larvae are found in large pools and swamps but the favoured breeding places throughout the year are artificial containers such as water butts and drainage pits. The larvae are tolerant of foul water.

TABLE 2.

Ratio of Length of the Upper Fork Cell to Its Petiole in the Female Wing. The Length of the Cell was taken as that of its Lower Branch.

	No.	Upper Fork Cell/Petiole.		
		Max.	Min.	Mean.
fatigans	50	3·7	2·5	3·2
molestus	50	8·5	4·4	5·2
australicus	50	4·1	2·6	3·2

c. *australicus.*

This is the mosquito referred to by Drummond (1951) as an undescribed member* of the *C. pipiens* complex in Australia. Previously it had been confused with *fatigans*, but, in fact, is more closely allied to *pipiens*.

It has a general dark colour, the basal tergal bands are constricted at the sides and the venter has prominent median and lateral patches of dark scales. It is, therefore, readily distinguished from *molestus* and, with typical specimens, from *fatigans* also. With material from any one locality *australicus* and *fatigans* can be separated by the differences in colour, but with specimens from different areas separation of females is sometimes impossible. The venational character, the ratio of the first fork cell to its petiole, which is useful for distinguishing *fatigans* from *molestus*, is of no value in separating *fatigans* and *australicus* (Table 2).

Males, however, can be reliably identified by the palps and the hypopygium. Characteristics of the palps of members of the *pipiens* complex are shown in Table 3.

In both the absolute and relative length of the palpal segments *australicus* is intermediate between *pipiens* and *fatigans* but is closer to *pipiens*. The distinctive feature of the palps of *australicus*, as is shown in the table, is the abundance of hairs on the shaft. The distal half is densely clothed with long hairs. In *fatigans* the hairs are sparse and disposed more towards the tip (Fig. 1). A further distinction, seen in

* This is the mosquito which in correspondence has been called "*fatigans* type B" and "long-siphoned *fatigans*".